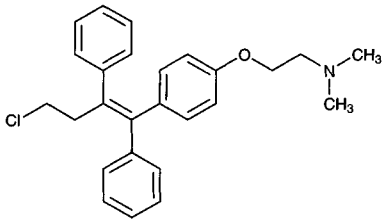
Toremifene



Molecular formula: $C_{26}H_{28}ClNO$
Molecular weight: 405.97
CAS Registry No.: 89778-26-7, 89778-27-8 (citrate)
Merck Index: 9688
Lednicer No.: 5 33

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL hexane:n-butanol 98:2, vortex for 1 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 300 μL MeOH, filter (0.2 μm). Place the filtrate in a quartz cuvette, irradiate with a mercury vapor lamp (15 W, peak wavelength 254 nm, General Electric No. G15T8) for 2 min (Caution! Protect personnel from UV radiation with aluminum foil shielding!), inject a 100 μL aliquot of the irradiated sample.

HPLC VARIABLES

Column: Ultrasphere ODS C18
Mobile phase: MeOH:water:triethylamine 92.9:7:0.1 (At the end of each day wash column with MeOH at 2 mL/min for 30 min.)
Flow rate: 2
Injection volume: 100
Detector: F ex 266

CHROMATOGRAM

Retention time: 6.03
Limit of detection: 8 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; UV irradiation; derivatization

REFERENCE

Holleran,W.M.; Gharbo,S.A.; DeGregorio,M.W. Quantitation of toremifene and its major metabolites in human plasma by high-performance liquid chromatography following fluorescent activation, *Anal.Lett.*, **1987**, *20*, 871–879.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 200 μL 2 μg/mL IS in MeCN, vortex for 10 s, centrifuge at 13000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: μBondapak C18
Column: 100 × 8 NovaPak C18 Rad-Pak
Mobile phase: MeCN:100 mM ammonium acetate:triethylamine 65:35:0.05 adjusted to pH 6.4 with acetic acid
Flow rate: 2
Injection volume: 100
Detector: UV 277

CHROMATOGRAM

Retention time: 5.4
Internal standard: (Z)-4-chloro-1,2-diphenyl-1-[4-[2-pyrroloethoxy]phenyl]-1-butene (Fc-1226a, Farmos) (6.1)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Webster,L.K.; Crinis,N.A.; Stokes,K.H.; Bishop,J.F. High-performance liquid chromatographic method for the determination of toremifene and its major human metabolites, *J.Chromatogr.*, **1991**, *565*, 482–487.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of serum to 9.5, extract 500 μL serum with diethyl ether. Remove the organic layer and evaporate it to dryness, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: reversed-phase C18
Mobile phase: MeOH:water 94:6 containing 18 mg/L diethylamine acetate
Flow rate: 2
Detector: F ex 267 em 377 following post-column photolysis in a PTFE coil

CHROMATOGRAM
Retention time: 7.5
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics; post-column photochemical derivatization

REFERENCE
Anttila,M.; Laakso,S.; Nyländen,P.; Sotaniemi,E.A. Pharmacokinetics of the novel antiestrogenic agent toremifene in subjects with altered liver and kidney function, *Clin.Pharmacol.Ther.*, **1995**, *57*, 628–635.

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Microsomal incubations. Add two volumes of MeOH:DMSO 80:20, centrifuge, inject an aliquot of the supernatant. Plasma. 200 μL Plasma + 400 μL MeOH:DMSO 80:20, vortex for 30 s, centrifuge at 5000 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil-ODS
Mobile phase: MeCN:250 mM pH 5.16 ammonium acetate buffer 65:35
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; liver; human

REFERENCE
Lim,C.K.; Yuan,Z.-X.; Ying,K.C.; Smith,L.L. High performance liquid chromatography of toremifene and metabolites, *J.Liq.Chromatogr.*, **1994**, *17*, 1773–1783.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 5 mL chilled chloroform to microsomal mixture, vortex, adjust aqueous phase to pH 9, extract with 5 mL chloroform. Combine the organic phases and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH:water 85:15, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-Select-B C8
Mobile phase: MeOH:water:triethylamine 80:20:0.01
Flow rate: 0.8
Detector: UV 238, or UV 277, or F ex 258 em 318 preceded by an on-line Knauer UV photoreactor

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Simultaneous: metabolites
Also analyzed: tamoxifen

KEY WORDS
post-column photochemical derivatization

REFERENCE
Berthou,F.; Dréano,Y. High-performance liquid chromatographic analysis of tamoxifen, toremifene and their major human metabolites, *J.Chromatogr.*, **1993**, *616*, 117–127.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 65:35 (Buffer was 100 mM ammonium acetate adjusted to pH 6.5 with glacial acetic acid.)
Flow rate: 1
Injection volume: 50
Detector: MS, VG Trio-2 quadrupole, thermospray, ion source 160°, vaporizer tip 160°, m/z 406

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 10 ng/mL

REFERENCE
Martinsen,A.; Gynther,J. Liquid chromatography-thermospray mass spectrometry of toremifene and its derivatives, *J.Chromatogr.A*, **1996**, *724*, 358–363.

SAMPLE
Matrix: urine
Sample preparation: 30 mL Urine + 20 mL 100 mM pH 8.5 phosphate buffer, extract twice with 60 mL portions of n-hexane:diethyl ether 90:10. Combine the extracts and evaporate them to dryness, reconstitute with 200 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax CN
Mobile phase: MeOH:100 mM pH 8 ammonium acetate 70:30
Flow rate: 1.2
Detector: UV 237 or MS, Hitachi M-80B double-focussing MS, API source (M-8093), nebulizer 350°, vaporizer 350°, needle current 15 μA, second electrode 3 keV, drift voltage 195 V, m/z 406

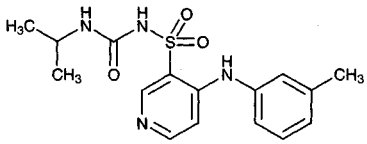
CHROMATOGRAM
Retention time: 19.5

Limit of detection: 50 ng

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Watanabe,N.; Irie,T.; Koyama,M.; Tominaga,T. Liquid chromatographic-atmospheric pressure ionization mass spectrometric analysis of toremifene metabolites in human urine, *J.Chromatogr.*, **1989**, *497*, 169–180.

Torsemide



Molecular formula: $C_{16}H_{20}N_4O_3S$
Molecular weight: 348.43
CAS Registry No.: 56211-40-6
Merck Index: 9690
Lednicer No.: 5 82

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL IS solution, mix, adjust pH to 4.5 with dilute HCl, add 4 mL ethyl acetate,, vortex for 1 min, centrifuge at 3000 rpm for 5 min, adjust pH of aqueous phase to 5, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL pH 10.2 buffer, add 4 mL ether, vortex, centrifuge. Acidify the aqueous phase to pH 4.5 and add 4 mL ethyl acetate, vortex, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 µL MeOH, inject a 2 µL aliquot. (Prepare IS solution by dissolving 30 mg IS in 10 mL 100 mM sodium bicarbonate solution, adjust pH to 7, make up to 25 mL with water.)

HPLC VARIABLES
Column: 250 × 2.6 ODS
Mobile phase: MeOH:water 30:70
Flow rate: 1
Injection volume: 2
Detector: UV 290

CHROMATOGRAM
Internal standard: 1-isopropyl-3-[[4-(4'-chloro-3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (JDL 487)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lesne,M.; Clerckx-Braun,F.; Duhoux,P.; van Ypersele de Strihou,C. Pharmacokinetic study of torasemide in humans: an overview of its diuretic effect, *Int.J.Clin.Pharmacol.Ther.Toxicol.*, **1982**, *20*, 382–387.

SAMPLE
Matrix: blood
Sample preparation: Condition a C2 SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 500 mM phosphoric acid. Condition a sulfonylpropyl ion-exchange SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 75 mM HCl. Add 1 mL plasma to the C2 SPE cartridge, add 50 µL 10 µg/mL IS in water, add 500 µL 500 mM phosphoric acid, air dry for 10 s, wash with 300 µL 500 mM phosphoric acid, elute with 1 mL MeOH:water 50:50, evaporate to dryness at 60° in a vortex evaporator for 30 min, reconstitute with 1 mL 75 mM HCl, add to the sulfonylpropyl SPE cartridge, wash with 1 mL dichloromethane, air dry, elute the contents of the SPE car-

tridge on to the column with mobile phase, after 5 min remove the SPE cartridge from the circuit.

HPLC VARIABLES
Guard column: C18
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:100 mM pH 4.5 KH_2PO_4 14:86 for 8 min, 30:70 for 3.5 min, 21:79 for 8.5 min (sic), 40:60 for 2 min, 21:79 for 10 min, 14:86 for 6 min (step gradients).
Column temperature: 45
Flow rate: 1.3
Detector: UV 290

CHROMATOGRAM
Retention time: 18.2
Internal standard: 1-isopropyl-3-[[4-(3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (28.0)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
March,C.; Farthing,D.; Wells,B.; Besenfelder,E.; Karnes,H.T. Solid-phase extraction and liquid chromatography of torsemide and metabolites from plasma and urine, *J.Pharm.Sci.*, **1990**, *79*, 453–457.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 100 μL IS solution, mix, adjust pH to 4.5 with pH 4 McIlvaine buffer, add 4 mL ethyl acetate, vortex for 1 min, centrifuge at 3000 rpm for 5 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL pH 11.8 buffer, wash twice with 4 mL portions of ether. Acidify the aqueous phase to pH 4.5 and extract twice with 4 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 50 μL MeOH, inject a 2 μL aliquot. (Prepare IS solution by dissolving 30 mg IS in 10 mL 100 mM sodium bicarbonate solution, adjust pH to 7, make up to 25 mL with water.)

HPLC VARIABLES
Column: 300 × 3.9 ODS
Mobile phase: MeCN:50 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1.5
Injection volume: 2
Detector: UV 290

CHROMATOGRAM
Internal standard: 1-isopropyl-3-[[4-(4'-chloro-3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (JDL 487)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; dog; pharmacokinetics

REFERENCE
Ghys,A.; Denef,J.; de Suray,J.; Gerin,M.; Georges,A.; Delarge,J.; Willems,J. Pharmacological properties of the new potent diuretic torasemide in rats and dogs, *Arzneimittelforschung*, **1985**, *35(II)*, 1520–1526.

SAMPLE
Matrix: formulations, urine
Sample preparation: Tablets. Pulverize and dissolve in MeOH, filter (0.45 μm membrane), dilute with mobile phase, inject a 20 μL aliquot. Urine. 5 mL Urine + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject a 20 μL aliquot (J. Chromatogr.A 1993, 655, 233. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 35:65 containing 5 mM KH_2PO_4/K_2HPO_4, pH 5.3
Flow rate: 1.0
Injection volume: 20
Detector: E, PAR Model 400, Ag/AgCl reference electrode, glassy carbon cell 1.3 V

CHROMATOGRAM
Retention time: 5.89
Limit of detection: 8.5 ng/mL

KEY WORDS
tablets

REFERENCE
Barroso,M.B.; Alonso,R.M.; Jiménez,R.M. Quantitative analysis of the loop diuretic torasemide in tablets and human urine by HPLC-EC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 179–186.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 10 μL 11.6 M perchloric acid, cool on ice, add 4 nmoles 4-methylumbelliferone, centrifuge at 3000 g for 5 min. Remove the supernatant and add it to 1.5 g ammonium sulfate, extract twice with dichloromethane:isopropanol 85.15. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-pak C18
Mobile phase: MeCN:10 mM pH 4.3 acetate buffer 13.5:86.5
Flow rate: 2
Injection volume: 50
Detector: UV 290

CHROMATOGRAM
Retention time: 18.9
Internal standard: 4-methylumbelliferone (7.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Miners,J.O.; Rees,D.L.P.; Valente,L.; Verones,M.E.; Birkett,D.J. Human hepatic cytochrome P450 2C9 catalyzes the rate-limiting pathway of torsemide metabolism, *J.Pharmacol.Exp.Ther.*, **1995**, *272*, 1076–1081.

SAMPLE
Matrix: urine

Sample preparation: Condition a C2 SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 500 mM phosphoric acid. Condition a sulfonylpropyl ion-exchange SPE cartridge (Analytichem) with 1 mL MeOH and 1 mL 75 mM HCl. Add 1 mL urine to the C2 SPE cartridge, add 700 μL 500 mM phosphoric acid, add 50 μL 10 μg/mL IS in water, wash with 1 mL 500 mM phosphoric acid, wash with 1 mL dichloromethane, elute with two 200 μL aliquots of MeOH, evaporate the eluate to dryness in a vortex evaporator, reconstitute with 1 mL 30 mM phosphoric acid, add to the sulfonylpropyl SPE cartridge, wash two 1 mL portions of water, elute with two 75 μL aliquots of MeOH:500 mM calcium chloride, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Guard column: C18
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:10 mM pH 4.5 KH_2PO_4 15:85 for 6 min, 34:66 for 2 min, 25:75 for 5 min (sic), 30:70 for 10 min, 15:85 for 7 min (step gradients).
Flow rate: 1
Injection volume: 50
Detector: UV 290

CHROMATOGRAM

Retention time: 19.0
Internal standard: 1-isopropyl-3-[[4-(3'-trifluoromethylphenylamino)pyridine]-3-sulfonyl]urea (27.1)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; pharmacokinetics

REFERENCE

March,C.; Farthing,D.; Wells,B.; Besenfelder,E.; Karnes,H.T. Solid-phase extraction and liquid chromatography of torsemide and metabolites from plasma and urine, *J.Pharm.Sci.*, **1990**, *79*, 453–457.

SAMPLE

Matrix: urine
Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN: water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

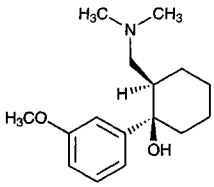
Retention time: 5.2
Internal standard: 7-propyltheophylline (4.5)

OTHER SUBSTANCES

Extracted: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, chlorthalidone, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, piretanide, polythiazide, probenecid, spironolactone, triamterene
Interfering: clopamide

REFERENCE
Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

Tramadol



Molecular formula: $C_{16}H_{25}NO_2$
Molecular weight: 263.38
CAS Registry No.: 27203-92-5, 22204-88-2 (HCl)
Merck Index: 9701
Lednicer No.: 2 17

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL pH 7.4 phosphate buffer at 6 mL/min. Centrifuge plasma at 4500 rpm for 15 min. Add 1 mL plasma to the SPE cartridge at 0.18 mL/min. Wash with 1 mL pH 7.4 phosphate buffer at 1.5 mL/min, elute with 150 μL MeOH at 1.5 mL/min and 350 μL pH 6.0 phosphate buffer containing 200 mM sodium perchlorate at 1.5 mL/min, mix the eluate, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 DIOL
Column: 250 × 4.6 10 μm Chiralcel OD-R, packed with cellulose tris-(3,5-dimethylphenylcarbamate) coated on silica
Mobile phase: MeCN:buffer 25:75 (Buffer was 50 mM phosphate buffer containing 200 mM sodium perchlorate, adjusted to pH 6.0 with NaOH solution.)
Column temperature: 30
Flow rate: 0.6
Injection volume: 200
Detector: UV 220; F ex 230 em 295

CHROMATOGRAM
Retention time: 13.3 (+), 14.9 (-)
Limit of detection: 500 pg/mL
Limit of quantitation: 1.5 ng/mL

OTHER SUBSTANCES
Extracted: active metabolite

KEY WORDS
plasma; chiral; SPE

REFERENCE
Ceccato,A.; Chiap,P.; Hubert,P.; Crommen,J. Automated determination of tramadol enantiomers in human plasma using solid-phase extraction in combination with chiral liquid chromatography, *J.Chromatogr.B*, **1997**, *698*, 161–170.

SAMPLE
Matrix: formulations
Sample preparation: Capsules. Dissolve the content of each capsule (ca. 50 mg) in 200 mL water, shake vigorously for 10 min and allow to settle down. Remove 10 mL supernatant, centrifuge at 6000 rpm for 5 min. Dilute 1 mL solution with water to a final concentration 10 μg/mL. Mix 150 μL aliquot with 150 μL 1 mg/mL metoclopramide in water. Inject a 50 μL aliquot. Intravenous ampoules. Dilute a 100 μL aliquot of the 100 mg/2 mL ampoule 100 fold with water to a final concentration 50 μg/mL. Mix 1 mL solution with 2.5 mL 1 mg/mL metoclopramide and make up to 10 mL with water. Inject a 75 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4 5 μm Bondapak C18
Column: 150 × 3.9 5 μm Bondapak C18
Mobile phase: MeCN:10 mM pH 5.5 sodium phosphate buffer containing 5 mM triethylamine 17:83
Flow rate: 1.2
Injection volume: 25-75
Detector: UV 230

CHROMATOGRAM
Retention time: 6.2
Internal standard: metoclopramide (4.4)
Limit of detection: 75 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
capsules; intravenous ampoules

REFERENCE
Zaghloul,I.Y.; Radwan,M.A. High performance liquid chromatographic determination of tramadol in pharmaceutical dosage forms, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 779–787.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 200 μL 2.5 μg/mL IS and 400 μL EtOH to 200 μL microsomal incubation. Mix on a whirlmix, centrifuge at 2250 g for 15 min, make the supernatant alkaline with 100 μL 25% ammonium hydroxide, add 5 mL dichloromethane, vortex for 1 min, centrifuge at 2500 g for 15 min. Discard the aqueous phase, evaporate the dichloromethane phase using nitrogen at 37°, reconstitute the residue in 200 μL EtOH:water 75:25 by agitating on a whirlmix for 30 s, inject an aliquot.

HPLC VARIABLES
Guard column: 125 × 3 Nucleosil RP 18
Column: 300 × 4 Nucleosil RP 18 (100-10)
Mobile phase: MeOH:100 mM ammonium hydrogen carbonate:25% ammonium hydroxide:triethylamine 50:49:1:0.01
Injection volume: 100
Detector: F ex 280, em 310

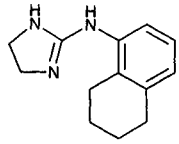
CHROMATOGRAM
Retention time: 64
Internal standard: 1-(*m*-hydroxyphenyl)-2-(N-ethyl-N-methylaminomethyl) cycloheptan -1-ol hydrochloride (35)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Paar,W.D.; Frankus,P.; Dengler,H.J. High-performance liquid chromatographic assay for the simultaneous determination of tramadol and its metabolites in microsomal fractions of human liver, *J.Chromatogr.B*, **1996**, *686*, 221–227.

Tramazoline



Molecular formula: $C_{13}H_{17}N_3$
Molecular weight: 215.30
CAS Registry No.: 1082-57-1, 3715-90-0 (H_2O)
Merck Index: 9702
Lednicer No.: 1 243

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 13.27

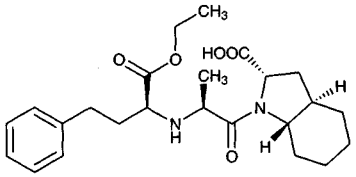
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Trandolapril



Molecular formula: $C_{24}H_{34}N_2O_5$
Molecular weight: 430.54
CAS Registry No.: 87679-37-6, 87679-71-8 (trandolaprilat)
Merck Index: 9703

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

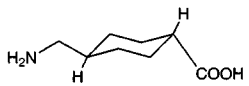
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 16.993

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tranexamic acid



Molecular formula: $C_8H_{15}NO_2$
Molecular weight: 157.21
CAS Registry No.: 1197-18-8
Merck Index: 9704
Lednicer No.: 2 9

SAMPLE
Matrix: blood
Sample preparation: 20 μL Serum + 2 μL water + 20 μL MeCN, mix, centrifuge at 10000 g for 3 min. Remove 5 μL of the supernatant and add it to 100 μL 25 mM pH 8 phosphate buffer, add 100 μL 300 μg/mL fluorescamine in acetone, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeCN:water:acetic acid:THF 30:69:0.5:0.5, containing 40 mM sodium acetate
Flow rate: 2
Injection volume: 20
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 5
Internal standard: tranexamic acid

OTHER SUBSTANCES
Extracted: 6-aminocaproic acid

KEY WORDS
serum; derivatization; tranexamic acid is IS

REFERENCE
Lacroix,C.; Levert,P.; Laine,G.; Goulle,J.P. Microdosage de deux antifibrinolytiques (acide β-aminocaproïque et acide tranexamique) par chromatographie liquide et détection fluorimétrique [Microanalysis of two antifibrinolytics (epsilon-aminocaproic acid and tranexamic acid) by liquid chromatography and fluorometry], *J.Chromatogr.*, **1984**, *309*, 183–186.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 250 μg/mL IS in water + 700 μL water, mix, add 200 μL 4 M perchloric acid, shake vigorously,let stand for 10 min, centrifuge at 3000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm cation-exchange (Brownlee)
Column: 250 × 4.6 10 μm Nucleosil SA
Mobile phase: MeOH:buffer 2:98 containing 100 μL/L caprylic acid (Buffer was 100 mM trisodium citrate adjusted to pH 4 with HCl.)
Column temperature: 26
Flow rate: 1.4
Injection volume: 100
Detector: F ex 410 em 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and the mixture flowed through a 1 m × 0.3 mm i.d. coil of tubing to the detector. (Prepare reagent by adding 800 mg o-phthalaldehyde in 10 mL MeOH to 1 L 700 mM pH 9.5 potassium borate buffer containing 2 g EDTA and 2 mL mercaptoethanol.)

CHROMATOGRAM
Retention time: 5.65
Internal standard: 4-aminomethyl bicyclo(2,2,2)octane-1-carboxylic acid (KabiVitrum, Uxbridge, UK) (6.65)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: arginine, histidine
Noninterfering: amino acids

KEY WORDS
post-column reaction; plasma

REFERENCE
Elworthy,P.M.; Tsementzis,S.A.; Westhead,D.; Hitchcock,E.R. Determination of plasma tranexamic acid using cation-exchange high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1985**, *343*, 109–117.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 5 μL 58.4 μg/mL IS, mix by swirling, add 2 mL EtOH, vortex, centrifuge at 1500 g for 10 min. Remove the supernatant and add it to 1 mL 10 mM pH 9.2 borax solution, add 13 μL phenylisothiocyanate, heat at 40° for 30 min, add 2 mL xylene, agitate, centrifuge at 1500 g for 10 min, wash twice more. Acidify the aqueous layer with 1 mL concentrated HCl, heat at 80° for 10 min, evaporate to dryness under reduced pressure, reconstitute with 1 mL 100 mM borax solution, extract twice with 2 mL portions of benzene (Caution! Benzene is a carcinogen!). Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil 5C8 (Nakarai Chemicals)
Mobile phase: EtOH:20 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.8
Internal standard: 3-aminocyclohexanecarboxylic acid (14.1)
Limit of detection: 200 ng/mL

KEY WORDS
serum; derivatization

REFERENCE
Matsubayashi,K.; Kojima,C.; Tachizawa,H. Determination of tranexamic acid in human serum by high-performance liquid chromatography using selective pre-column derivatization with phenyl isothiocyanate, *J.Chromatogr.*, **1988**, *433*, 225–234.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out powdered tablet containing aminocaproic acid, dissolve in 100 mL water, filter (0.45 μm). Mix a 5 mL aliquot of the filtrate with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. Injections, syrup. Weigh out amount of injection or syrup containing 250 mg aminocaproic acid, dilute with 100 mL water, dilute an aliquot 5-fold with water. Mix a 5 mL aliquot with 10 mL 5 mg/mL dansyl chloride in acetone and 10 mL 400 μg/mL tranexamic acid in buffer, let stand in the dark at room temperature for 30 min, add 2 drops ethanolamine, mix, let stand at room temperature for 15 min, make up to 50 mL with acetone:water 50:50, mix, inject an aliquot. (Prepare buffer by dissolving 550 mg anhydrous sodium carbonate in 300 mL water, add 300 mL acetone, mix.)

HPLC VARIABLES
Guard column: C18 (Alltech)
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: MeOH:water:acetic acid:triethylamine 60:38:1.5:0.5
Flow rate: 1.5
Injection volume: 20
Detector: UV 335

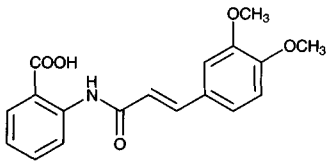
CHROMATOGRAM
Retention time: 6.5
Internal standard: tranexamic acid

OTHER SUBSTANCES
Simultaneous: aminocaproic acid

KEY WORDS
derivatization; tablets; injections; syrup; tranexamic acid is IS

REFERENCE
Lau-Cam,C.A.; Roos,R.W. Assay of aminocaproic acid in dosage forms by reversed phase high performance liquid chromatography with dansylation, *J.Liq.Chromatogr.*, **1993**, *16*, 403–419.

Tranilast



Molecular formula: $C_{18}H_{17}NO_5$
Molecular weight: 327.34
CAS Registry No.: 53902-12-8
Merck Index: 9705

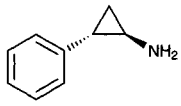
SAMPLE
Matrix: tissue
Sample preparation: Wipe the skin with liquid paraffin and EtOH. Separate the tissue by a heat separation technique, mince with scissors, homogenise, add MeOH. Centrifuge, inject an aliquot of supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Inertsil ODS-2
Mobile phase: MeOH:0.1% phosphoric acid 75:25
Flow rate: 1.0
Detector: UV 320

KEY WORDS
skin; Yucatan micropig; pig

REFERENCE
Hori,N.; Fujii,M.; Yamanouchi,S.; Miyagi,M.; Saito,N.; Matsumoto,M. In vitro release of tranilast from oily gels and penetration of the drug into Yucatan micropig skin, *Biol.Pharm.Bull.*, **1998**, *21*, 300–303.

Tranylcypromine



Molecular formula: $C_9H_{11}N$
Molecular weight: 133.19
CAS Registry No.: 155-09-9, 13492-01-8 (sulfate)
Merck Index: 9708
Lednicer No.: 1 73

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 μL 100 μg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 μL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM
Retention time: 9.0 (S-(-)), 10.7 (R-(+))

OTHER SUBSTANCES
Extracted: amphetamine
Interfering: methamphetamine

KEY WORDS
plasma; derivatization; normal phase; chiral

REFERENCE
Weber,H.; Spahn,H.; Mutschler,E.; Möhrke,W. Activated α-alkyl-α-arylacetic acid enantiomers for stereoselective thin-layer chromatographic and high-performance liquid chromatographic determination of chiral amines, *J.Chromatogr.*, **1984**, *307*, 145–153.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 500 ng/mL apomorphine in 1 mM phosphoric acid + 1 mL buffer + 5 mL n-heptane:n-octanol:tetraoctylammonium bromide 89.75:10:0.25, shake

by hand for 2 min, centrifuge at 4° at 1500 g for 5 min. Remove 4 mL of the organic layer and add it to 4 mL n-octanol and 1 mL 50 mM phosphoric acid, shake by hand for 2 min, centrifuge at 4° at 1500 g for 5 min, inject a 100 μL aliquot of the aqueous layer. (Buffer was 2 M pH 8.45 ammonium chloride/ammonium hydroxide buffer containing 0.2% diphenylborate ethyleneamine and 0.5% EDTA.)

HPLC VARIABLES

Column: 150 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 100 mM NaH_2PO_4 containing 0.3 g/L NaCl and 0.76 g/L sodium 1-octanesulfonate, pH adjusted to 3 with phosphoric acid.)
Flow rate: 0.5
Injection volume: 100
Detector: E, Spark-analytica model 9205, 1.0 V

CHROMATOGRAM

Retention time: 12.05
Internal standard: apomorphine (13.81)
Limit of detection: 5 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Krugers Dagneaux,P.G.L.C.; Loohuis,C.P.G.G.; Klein Elhorst,J.T.; Van der Veer,T.S. Liquid chromatographic estimation of tranylcypromine in human plasma, *Pharm.Weekbl.[Sci].*, **1992**, *14*, 46–49.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 1.43
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, mianserin, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: moclobemide, pentobarbital, metoclopramide

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL pH 11 sodium borate buffer, extract with 2.5 mL diisopropyl ether:EtOH 100:1.5 (Caution! Diisopropyl ether readily forms explosive peroxides!). Remove 2 mL of the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 200 μL 10 μg/mL S-flunoxaprofen chloride, let stand at room temperature for 1 h, add 20 μL MeOH, evaporate to dryness, reconstitute with dichloromethane, inject a 5-50 μL aliquot. Urine. 1 mL Urine + 1 mL 50 mM NaOH, extract with 2.5 mL diisopropyl ether:EtOH 100:1.5. Remove 2 mL of the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 200 μL 10 μg/mL S-flunoxaprofen chloride, let stand at room temperature for 1 h, add 20 μL MeOH, evaporate to dryness, reconstitute with dichloromethane, inject a 5-50 μL aliquot. (Prepare S-flunoxaprofen chloride as follows. Dissolve 1 mmole S-flunoxaprofen in 25 mL toluene, add a trace of DMF (J.Chromatogr. 1990, 528, 55), add 2.5 mL thionyl chloride, reflux for 30 min, remove solvent by evaporation, dry the residue under vacuum over KOH, recrystallize from dichloromethane (mp 73°).)

HPLC VARIABLES
Guard column: 4 × 4 LiChrosorb Si 60
Column: 250 × 4.6 7 μm Zorbax Sil
Mobile phase: Cyclohexane:dichloromethane:THF 70:10:10
Flow rate: 1.4
Injection volume: 5-50
Detector: F ex 305 em 355

CHROMATOGRAM
Retention time: 12 (S-(-)), 15 (R-(+))
Limit of detection: 2 ng/mL

KEY WORDS
normal phase; derivatization; chiral; plasma

REFERENCE
Spahn,H. *S*-(+)-Flunoxaprofen chloride as chiral fluorescent reagent, *J.Chromatogr.*, **1988**, *427*, 131–137.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or 500 μL urine + 1 mL 100 mM pH 11 sodium borate buffer + 20 μL 1 (plasma) or 10 (urine) μg/mL S-(+)-amphetamine in MeOH + 5 mL diethyl ether: EtOH 98.5:1.5, mix for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL reagent, vortex briefly, let stand at room temperature for 5 min, inject an aliquot. (Reagent was 10 mg o-phthaldialdehyde, 500 μL EtOH, and 40 mg N-acetyl-L-cysteine in 5 mL buffer. Buffer was 14.75 g boric acid and 160 mL 1 M NaOH made up to 1 L with water, pH 10.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:THF:buffer 60:1:50 (Buffer was 653 mL 9.07 g/L KH_2PO_4 and 347 mL 11.87 g/L $Na_2HPO_4.2H_2O$.)
Flow rate: 1.2
Detector: F ex 344 em 442

CHROMATOGRAM
Retention time: 32.5 (S-(-)), 35 (R-(+))

Internal standard: S-(+)-amphetamine (25)
Limit of detection: 2 ng/mL (urine), 0.5 ng/mL (plasma)

OTHER SUBSTANCES
Noninterfering: norephedrine, norepinephrine, norpseudoephedrine, tyramine

KEY WORDS
plasma; chiral; derivatization; pharmacokinetics

REFERENCE
Spahn-Langguth,H.; Hahn,G.; Mutschler,E.; Möhrke,W.; Langguth,P. Enantiospecific high-performance liquid chromatographic assay with fluorescence detection for the monoamine oxidase inhibitor tranylcypromine and its applicability in pharmacokinetic studies, *J.Chromatogr.*, **1992**, *584*, 229–237.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.66

OTHER SUBSTANCES
Simultaneous: levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.8

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax Sil
Mobile phase: n-Hexane:THF:isopropanol 83:12:5
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM
Retention time: 19.6 (-), 21.6 (+)

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions
Sample preparation: Mix sample:50 (?) mM NaCN in 50 mM pH 9.3 borate buffer:25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was THF:50 mM pH 6.8 potassium phosphate buffer 5:95. B was MeCN:MeOH:50 mM pH 6.8 potassium phosphate buffer 55:10:35. A:B from 70:30 to 0:100 over 1 h, maintain at 0:100 for 20 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 68

OTHER SUBSTANCES
Simultaneous: baclofen, amphetamine

KEY WORDS
derivatization

REFERENCE
Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE
Matrix: solutions
Sample preparation: 50 μL 5 mg/mL Tranylcypromine in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 16.3 mg N-acetyl-L-cysteine in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:MeCN:buffer 50:2:50 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 425 or UV 254

CHROMATOGRAM
Retention time: 25.81 (first enantiomer) (α = 1.13)

KEY WORDS
derivatization; protect from light; chiral; α = 1.13

REFERENCE
Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 7.0

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate
Interfering: tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine,

chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Mix 1 mL urine with 1 mL 200 mM boric acid/KCl buffer, adjust pH to 8.5, add 5 mL n-hexane:n-octanol 90:10, invert repeatedly for 2 min, centrifuge at 1500 g for 15 min. Remove the organic layer and extract it further with 4 mL n-octanol and 1 mL 50 mM phosphoric acid, in the same manner. Centrifuge, discard the organic layer, inject a 20 µL aliquot of aqueous phase

HPLC VARIABLES

Column: 150 × 3.9 5 µm Symmetry C18
Mobile phase: MeOH:water:50 mM pH 4.55 KH_2PO_4:70:20
Column temperature: 23
Flow rate: 1
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 6.28
Limit of detection: 5 nmol/mL
Limit of quantitation: 25 nmol/mL

REFERENCE
Aboul-Enein,H.Y.; Abou-Basha,L.I. Determination of tranylcypromine in urine and pharmaceutical formulation by HPLC using symmetry column, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 925–932.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 1 mL urine to 8.5 with 1 mL 200 mM boric acid KCl buffer, add 5 mL n-hexane:n-octanol 90:10, invert repeatedly for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and add it to 4 mL n-octanol and 1 mL 50 mM phosphoric acid, extract, centrifuge, inject a 20 μL aliquot of the aqueous layer.

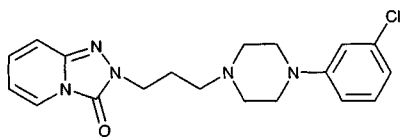
HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR (+) (Daicel)
Mobile phase: MeOH:100 mM perchloric acid 12:88
Column temperature: 10
Flow rate: 0.6
Injection volume: 20
Detector: UV 256

CHROMATOGRAM
Retention time: 12.93 (R-(+)), 16.38 (S-(-))

KEY WORDS
chiral

REFERENCE
Aboul-Enein,H.Y.; Serignese,V. Direct separation of tranylcypromine enantiomers and their profile in an atypical depressive patient, *Biomed.Chromatogr.*, **1995**, *9*, 98–101.

Trazodone



Molecular formula: $C_{19}H_{22}ClN_5O$
Molecular weight: 371.87
CAS Registry No.: 19794-93-5, 25332-39-2 (HCl)
Merck Index: 9712
Lednicer No.: 2 472

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 3.0

Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, encainide, fluoxetine, flurazepam, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: diphenhydramine, doxepin, fentanyl, flecainide, haloperidol, nordoxepin

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 249

CHROMATOGRAM

Retention time: 4.98

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam;

tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: 50 µL Plasma or CSF + 20 µL 4 M NaOH, vortex briefly, add 750 µL diethyl ether, vortex for 1 min, centrifuge at 2600 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 25 × 4 Hibar LiChroCART C8 (Merck)
Column: 250 × 4 5 µm LiChrospher 100 CH-8 II C8
Mobile phase: MeCN:10 mM pH 3.0 phosphate buffer 60:40 containing 20 mM tetramethylammonium chloride
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 6.0
Internal standard: trazodone

OTHER SUBSTANCES

Extracted: toloxatone

KEY WORDS

plasma; rabbit; trazodone is IS

REFERENCE

Vistelle,R.; Lamiable,D.; Zinzou,M. Simple high-performance liquid chromatographic method for the measurement of toloxatone in rabbit cerebrospinal fluid and plasma, *J.Chromatogr.*, **1989**, *490*, 387–394.

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: Tissue homogenates were 1:2 in water. 1 mL Sample + 1 mL saturated sodium borate buffer + 100 μL 20 μg/mL methyl clonazepam in water + 5 mL n-butyl chloride, rotate at 40 rpm for 30 min, centrifuge at 2500 rcf for 5 min. Remove the organic phase and evaporate it to dryness at 70 ° under a stream of air, reconstitute the residue in 300 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 Analytichem ODS with an integral guard column
Mobile phase: MeCN:100 mM KH_2PO_4 300:700, adjust pH to 3.00 with concentrated phosphoric acid
Column temperature: 60
Flow rate: 1.5
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 1.25
Internal standard: methyl clonazepam (5.36)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: diazepam, temazepam
Also analyzed: acetaminophen, alprazolam, amitriptyline, amoxapine, carbamazepine, chlordiazepoxide, chlorpromazine, chlorprothixene, clonazepam, demoxepam, desipramine, diphenhydramine, disopyramide, doxepin, ethotoin, flurazepam, glutethimide, haloperidol, haloperidol, imipramine, lidocaine, lorazepam, loxapine, maprotiline, mesantoin, mesoridazine, methaqualone, methotrimeprazine, nordiazepam, nortriptyline, oxazepam, pentazocine, perphenazine, phenacetin, phenobarbital, phenytoin, promazine, promethazine, propranolol, protriptyline, salicylic acid, thiothixene, trifluoperazine, triflupromazine, trimipramine
Noninterfering: thioridazine, chloral hydrate, codeine, ketamine, meperidine, methamphetamine, methypyrlon, methadone

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KEY WORDS
serum; plasma; whole blood

REFERENCE
Root,I.; Ohlson,G.B. Trazodone overdose: report of two cases, *J.Anal.Toxicol.*, **1984**, *8*, 91–94.

SAMPLE
Matrix: blood, gastric contents, tissue, urine
Sample preparation: Blood, stomach contents. 1 mL Postmortem blood or stomach contents + 500 μL 1 M potassium carbonate + 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot. Urine. 1 mL Urine + 200 μL concentrated HCl, heat at 100° for 1 h, cool, adjust pH to 9.5-10 with KOH pellets and 1 M potassium carbonate, add 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot. Tissue. Add tissue to an equal volume isotonic saline, homogenize with an Ultra-Turraz mixer, remove a 2 g aliquot, add 8 mL n-hexane:ethyl acetate 7:3, extract on a rotary mixer for 8 min, centrifuge at 2500 rpm for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.1 Chrompack pellicular reverse phase
Column: 100 × 3 5 μm Chromspher C8
Mobile phase: Gradient. MeOH:water containing 0.125% isopropylamine from 30:70 to 75:25 over 15 min
Flow rate: 0.7
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: dothiepin

REFERENCE
Lambert,W.; Van Bocxlaer,J.; Piette,M.; De Leenheer,A. A fatal case of trazodone and dothiepin poisoning: toxicological findings, *J.Anal.Toxicol.*, **1994**, *18*, 176–179.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 10 μL 20 μg/mL bupropion in MeOH, vortex briefly, add 500 μL saturated sodium borate, vortex briefly, add 5 mL MTBE, vortex briefly then mix on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min. Remove the organic phase and add it to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer. Tissue. Weigh whole brain and homogenize with 10 mL 340 mM perchloric acid containing 0.01 mM EDTA for 20 s (Brinkman PT 10/35). Remove a 1 mL aliquot and add 5 μL 20 μg/mL bupropion in MeOH, 500 μL 600 mM sodium carbonate, and 3 mL hexane:isoamyl alcohol 98:2 to it. Shake on a reciprocating shaker for 10 min, centrifuge at 220 g for 10 min, remove the organic layer and repeat the extraction. Combine the organic layers and add them to 75 μL 10 mM phosphoric acid, vortex, centrifuge, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm IBM reverse phase (trimethyl silane)
Mobile phase: MeCN:pH 3.0 phosphate buffer 27:73 containing 20 mM heptanesulfonic acid and 40 mM triethylamine
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 7.1
Internal standard: bupropion (10.6)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; rat; brain

REFERENCE
Miller,R.L.; DeVane,C.L. Analysis of trazodone and m-chlorophenylpiperazine in plasma and brain tissue by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 388–393.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)

Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 3.48
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, sulforidazine, thioridazine, thiothixene, tranylcypromine, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: nomifensine, quinine, quinidine, nordoxepin, norfluoxetine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 1.2 U/I β-glucuronidase in 200 mM pH 5.5 phosphate buffer, incubate at 37° for 2 h, add 100 μL 100 μg/mL sodium carbonate:sodium bicarbonate 1:1, extract three times with 2 mL dichloromethane:ethylene chloride:ethyl acetate 1:1:8, centrifuge at 8000 rpm for 5 min, dry organic layers over anhydrous sodium sulfate, evaporate to dryness at 35°/15 mmHg. Dissolve residue in 500 μL MeOH, vortex for 2 min, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:10 mM pH 7.5 phosphate buffer 12.5:67:20.5
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 600 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; rabbit

REFERENCE
di Tella,A.S.; Di Nunzio,C.; Ricci,P.; Parisi,G. Determination of trazodone and its metabolite, m-CPP, in serum and urine by HPLC, *J.Anal.Toxicol.*, **1986**, *10*, 233–235.

SAMPLE
Matrix: blood, urine

Sample preparation: Make 1 mL serum or urine basic (pH 12.8), extract into 5 mL diethyl ether. Remove the organic layer and evaporate it under a stream of nitrogen at room temperature, dissolve the residue in 250 μL 5 mM sulfuric acid, inject an aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm Spherisorb 5 S ODS
Mobile phase: MeCN:50 mM sulfuric acid 18:1
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 11.4
Internal standard: 2-[3-(4-m-chlorophenyl-1-piperazinyl)propyl]-5-methyl-4-phenyltriazol-3-(2H)-one (9.6)
Limit of detection: 25 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Nilsen,O.G.; Dale,O. Single dose pharmacokinetics of trazodone in healthy subjects, *Pharmacol.Toxicol.*, **1992**, *71*, 150–153.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 12.683

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.4

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)
Mobile phase: MeCN:20 mM pH 3.2 KH_2PO_4 23.4:76.6 containing 0.05% nonylamine
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: amitriptyline, desmethyldoxepin, desipramine, doxepin, imipramine, loxapine, maprotiline, nortriptyline

REFERENCE
Supelco Catalog, **1993,** p. 440.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.37 (A), 4.58 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.